Supporting Information

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SI Text

Animals. All animal experiments were performed according to procedures approved by the Institutional Animal Care and Use Committee. Endurance exercise was measured using in-cage voluntary running wheels, with electronic monitoring (VitalView), as described in the text. All mice were house individually. Unless otherwise indicated, 8-week-old mice were used for all experiments. Whole body and skeletal-muscle-specific PGC-1α $-/-$ mice have been described (1, 2), as have ERRα $-/-$ mice (3) and MCK-PGC-1α mice (4).

Cells and Reagents. All reagents were from Sigma, unless otherwise indicated. Immunostaining was performed using anti-CD31 antibody (BD PharMingen). Quantification of capillaries was performed computationally, using Volocity software (Improvision, Perkin-Elmer), on three random fields chosen from the midportions of transverse sections from quadriceps. All quantifications were performed blindly. Isolation and culture of primary skeletal myocytes was performed as described in ref. 5. Cells were infected with adenovirus at a multiplicity of infection of 10–30, and mRNA expression was measured 48 h later. The adenovirus expressing PGC-1α has been described (6). Reporter plasmids containing VEGF enhancer, mutated enhancer, and concatemerized ERRα binding sites have been described (7, 8).

Gene Expression Studies. Total RNA's were isolated from mouse tissue or cultured cells using the TRIzol method (Invitrogen). Samples for real-time PCR analyses were reverse transcribed (Invitrogen), and quantitative real-time PCRs were performed on the cDNA's in the presence of fluorescent dye (SYBR green, ABI), on an ABI 7300 machine. The alternative PGC-1α promoter-luciferase plasmid was generated by amplifying a 4-kilobase fragment of DNA encompassing 3.7 kb 5′ to 300 bases 3′ of the alternative start site and subcloning into the PGL3basic plasmid (Promega). Subsequent deletions were generated using site-directed mutagenesis (Stratagene). The proximal PGC-1α promoter-luciferase plasmid has been described (8). In vivo transfections were performed as described (9–12). Three to 10 days later, luciferase activity in muscle extracts was measured using the Dual-Luciferase Reporter Assay (Promega). Genome analyses were performed using the UCSC genome browser, http://genome.ucsc.edu/ (13). Analyses for conserved transcription factor binding sites were performed using the ECR browser at http://ecrbrowser.dcode.org/ (14). All results are expressed as means ± SEM. Two-tailed independent Student's t tests were used to determine all P values.

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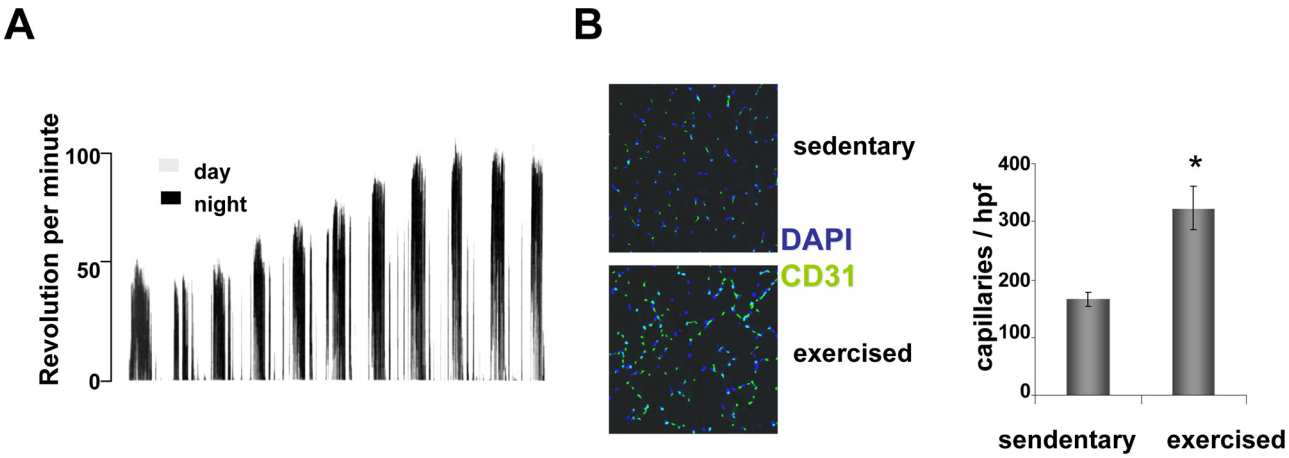

Fig. S1. Voluntary endurance running induces angiogenesis in skeletal muscle. (*A*) Eight-week-old c57/Bl6 mice were individually housed in cages containing electronically monitored voluntary running wheels. A sample tracing of wheel activity, in revolutions per minute, is shown. Day and night are indicated in white and black, respectively. (*B*) Immunostaining of midportion of transverse sections of quadriceps muscles from animals after the indicated number of days of voluntary running. *Left*, sample high-power fields from exercised animals (14 days) and sedentary controls. *Right*, quantification of capillary density, $n = 4$ per group. Data are presented as mean ± SEM. $*P < 0.05$ vs. day 0.

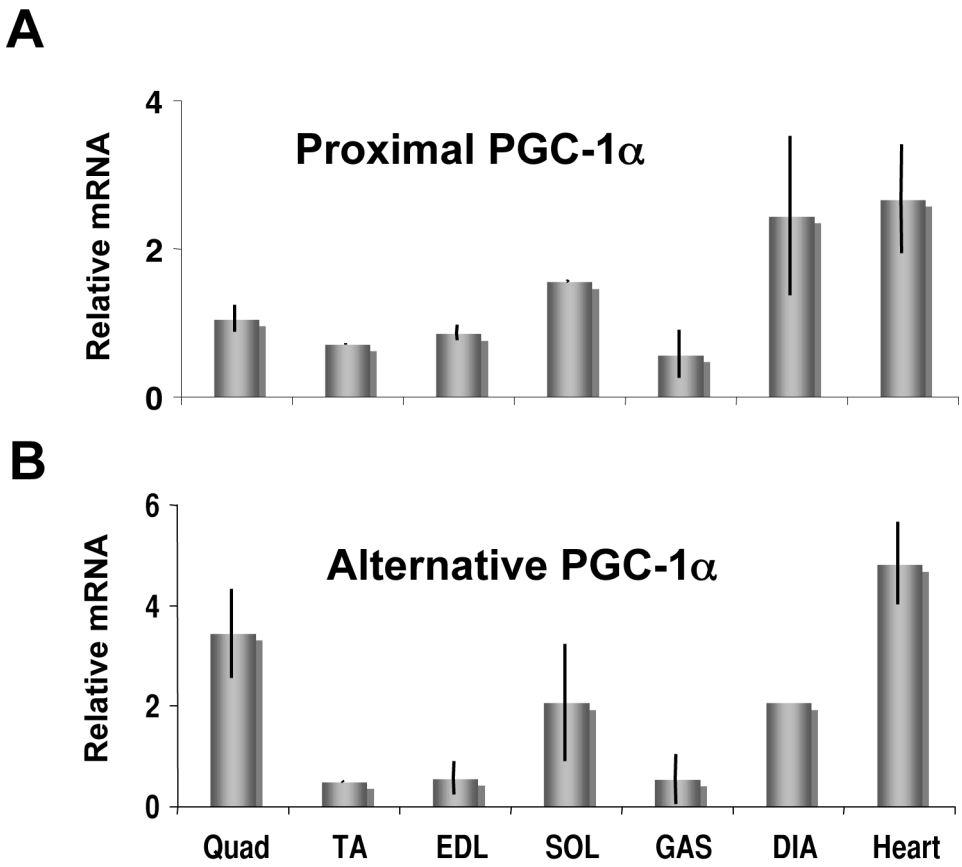

Fig. S2. Relative expression of PGC-1α mRNA originating at the proximal promoter (*A*), or the alternative promoter (*B*), in the indicated muscle beds, as determined by qPCR. Quad, quadriceps; TA, tibialis anterior; EDL, extensor digitorum longus; SOL, soleus; GAS, gastrocnemius; DIA, diaphragm.

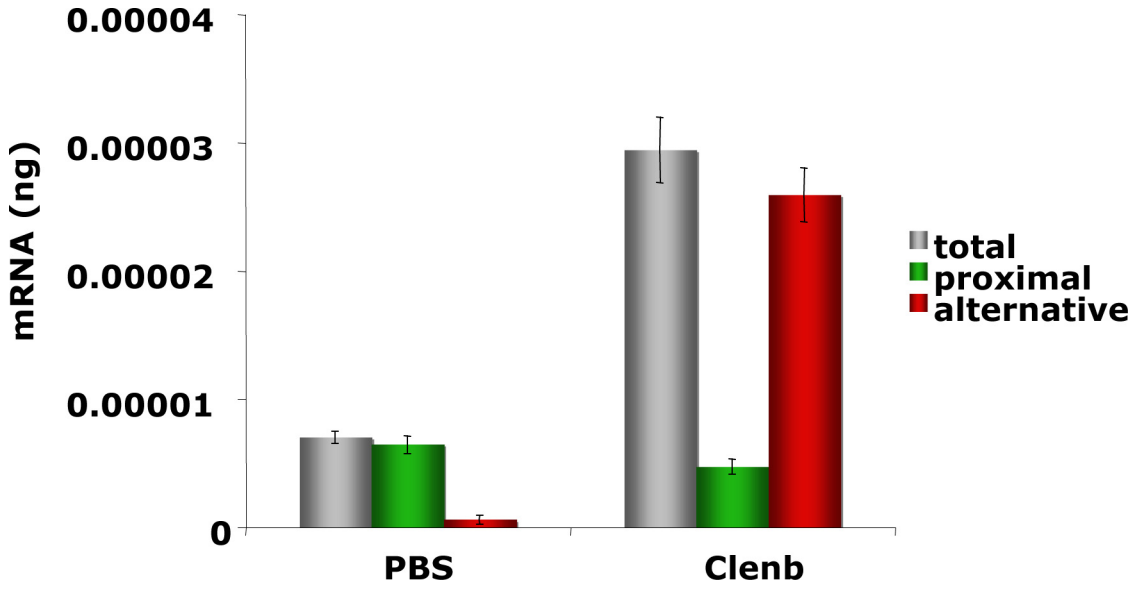

Fig. S3. Absolute expression in quadriceps of total PGC-1α mRNA (gray), PGC-1α mRNA originating at the proximal promoter (green), or the alternative promoter (red), 6 h after PBS or clenbuterol injection. Absolute expression levels were determined by qPCR and standardization curves using known standards.

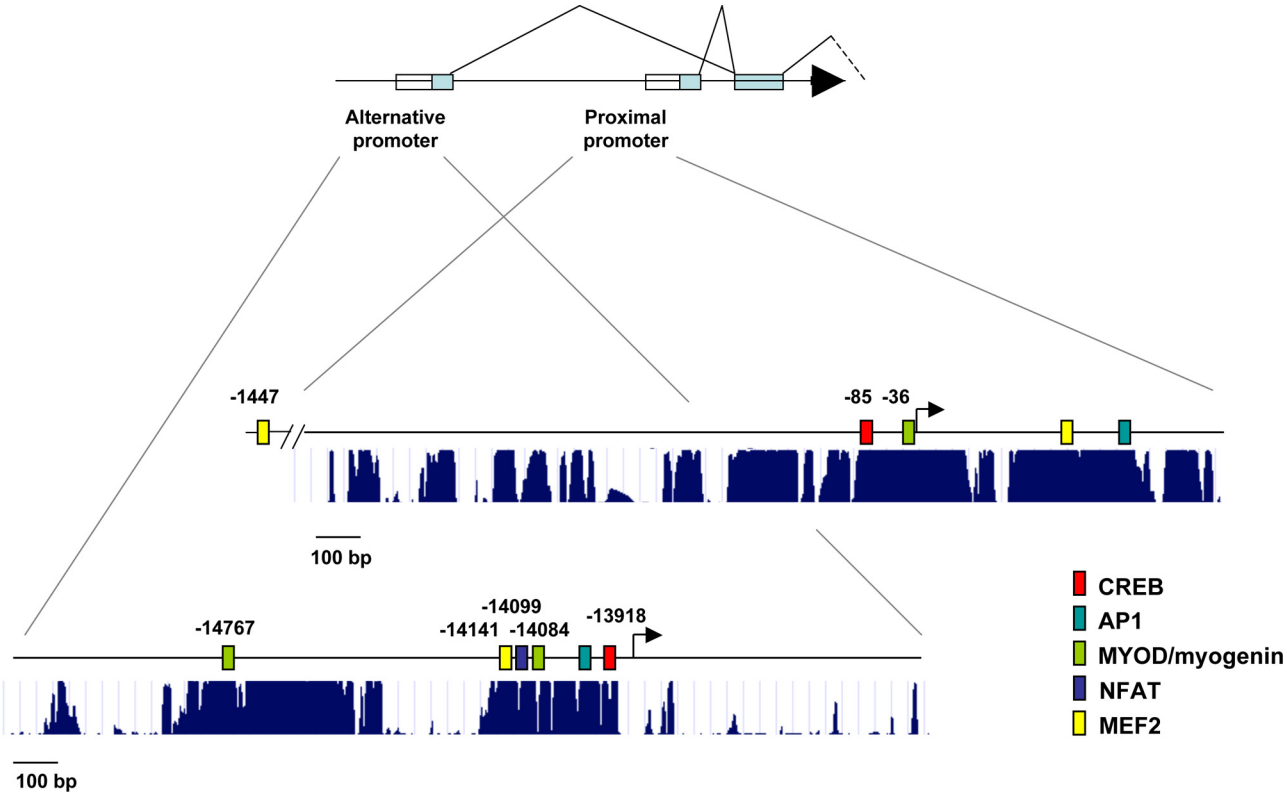

Fig. S4. Comparison of the proximal and alternative PGC-1α promoters. Level of mammalian conservation is shown in blue. Small arrows indicate transcriptional start sites. Conserved consensus transcription factor binding sites are indicated in colored boxes.

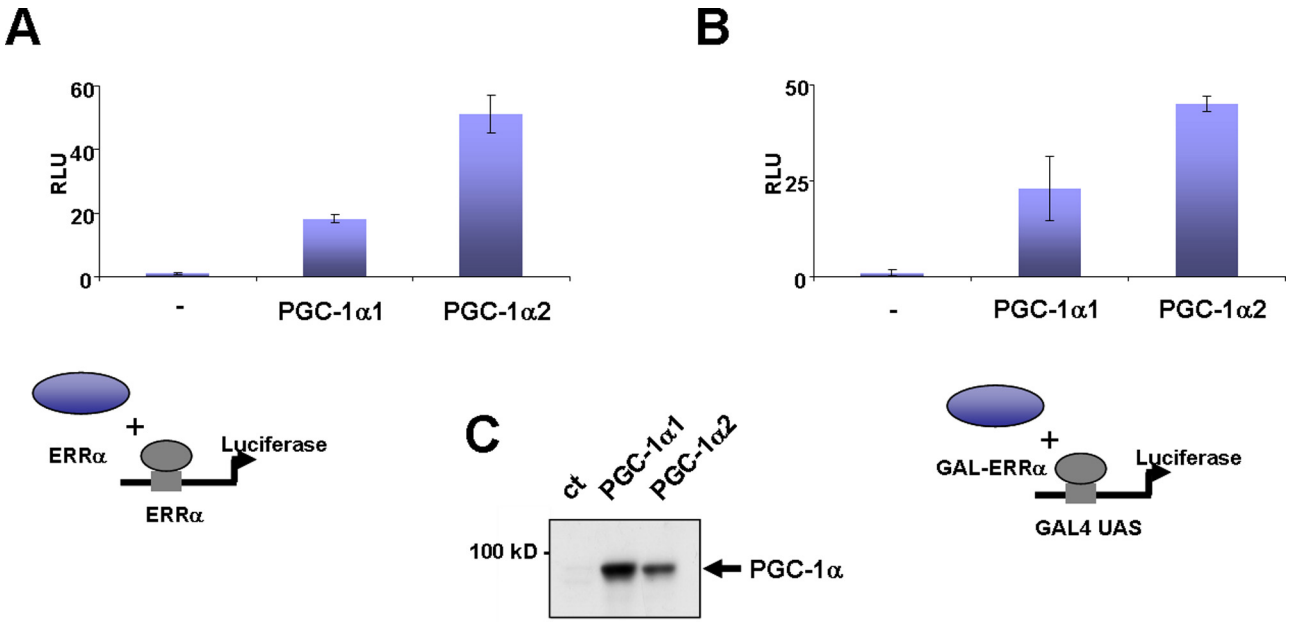

Fig. S5. PGC-1α coactivates ERRα. (*A*) Plasmids encoding for ERRα and the indicated PGC-1α were cotransfected with a reporter plasmid containing concatemerized ERRα binding sites driving a luciferase gene. After 24 h, Luciferase activity was measured, and shown as relative light units (RLU). (*B*) Plasmids encoding for a GAL4-ERRα fusion protein and the indicated PGC-1α were cotransfected with a reporter plasmid containing concatemerized GAL4 binding sites driving a luciferase gene. After 24 h, Luciferase activity was measured, and shown as relative light units (RLU). (*C*) Western blot analysis of PGC-1α1 and PGC-1α2 in the same cells as in *A* and *B*.

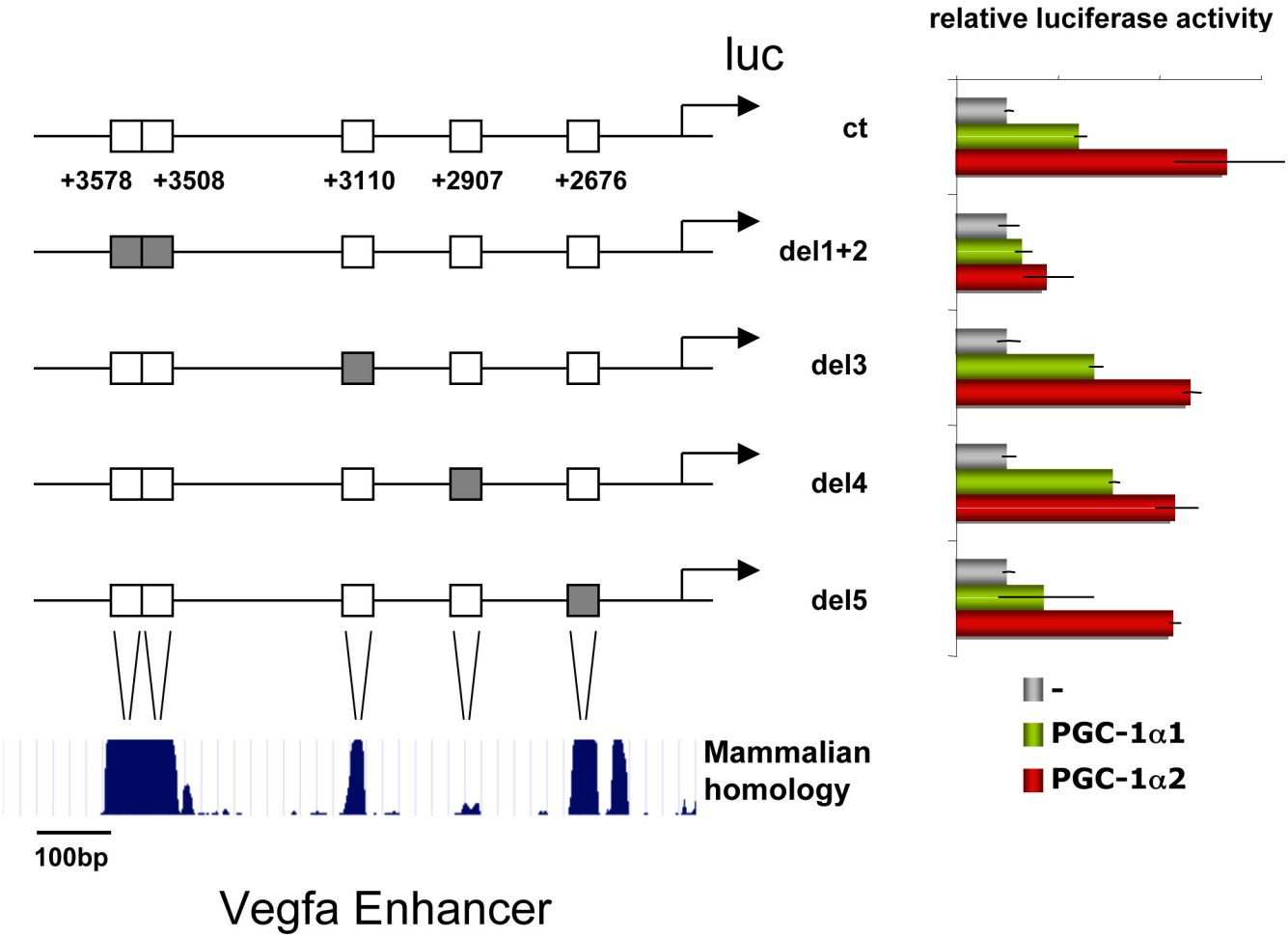

Fig. S6. PGC-1α2 coactivates ERRα on the VEGF enhancer. Plasmids encoding for ERRα and the indicated PGC-1α were cotransfected with either an intact reporter plasmid containing the VEGF enhancer and the SV40 promoter driving a luciferase gene, or the same construct bearing mutations in the indicated conserved ERRα binding sites (schematized as small boxes). After 24 h, Luciferase activity was measured, and shown as relative light units.